Human Vascular Microphysiological System for *in vitro* Drug Screening

Supplementary Information

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Supplementary Materials and Methods:

S1. Tensile Testing of TEBVs

Circumferential tensile strength was analyzed using a micro-strain analyzer (TA Instruments) with modified grips as shown in Fig. S1. TEBVs were cut in 5 mm sections and mounted through grips with diameters of 300 μm. Cyclic pre-conditioning was performed by stretching the lumen to a strain of 20% for 6 cycles (*1*). An optimized strain rate of 0.08 mm/s was used to stretch rings until failure.

Grip diameters were taken into account in calculating the strain (ε), where D = grip diameter. Stress (σ) was calculated by assuming conservation of volume.

$$\varepsilon = \frac{L_i - L_s}{L_s};\ L_s = 2D + L_0$$

$$\sigma = \frac{F}{A}\left(1 + \frac{\Delta L}{L_0}\right)$$

Ultimate tensile stress was defined as the maximum stress before failure. The Young's modulus (E) was calculated by dividing stress by strain.

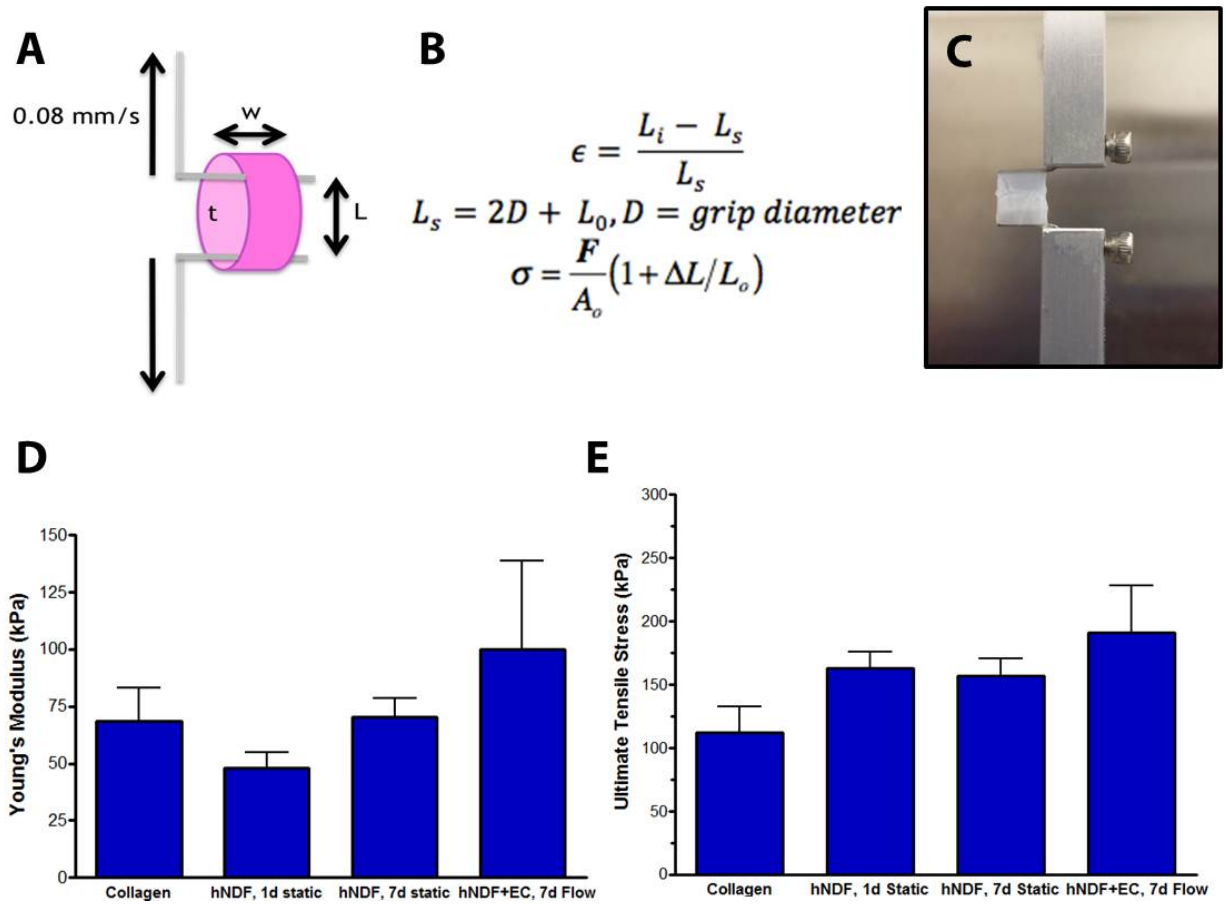

Fig S1: TEBV Mechanical Testing. TEBVs were cut into rings approximately 5 mm in width (w) and mounted on modified grips (**A**). Stress and strain were calculated by taking the diameter change into account as the TEBV was pulled in tension (**B**). TEBVs stretched significantly throughout the mechanical test (**C**). Mechanical stimulation had a demonstrated impact on TEBV Young's modulus (**D**), and ultimate tensile stress (**E**). Data shown as mean ± SEM, n = 3-7 TEBVs.

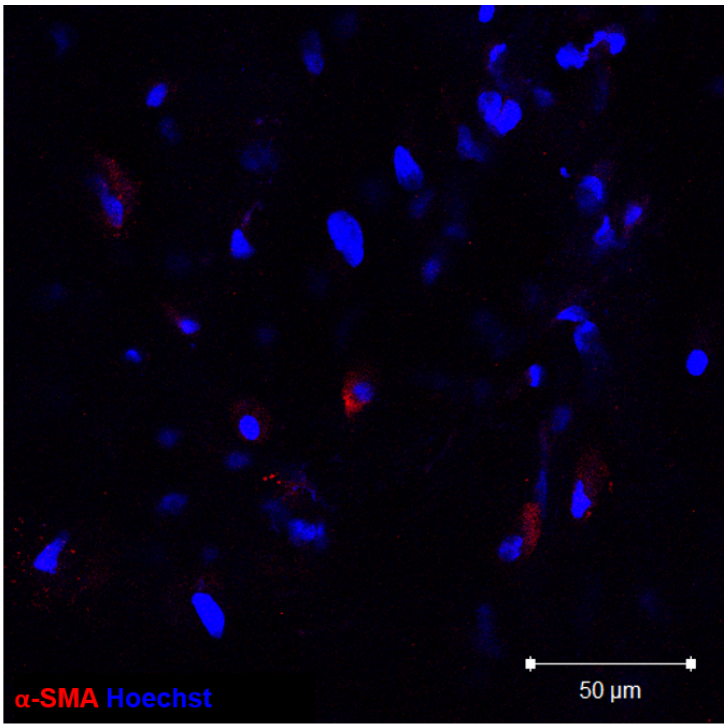

Fig. S2: Expression of α-SMA in hNDF TEBVs after 24 hours of culture.

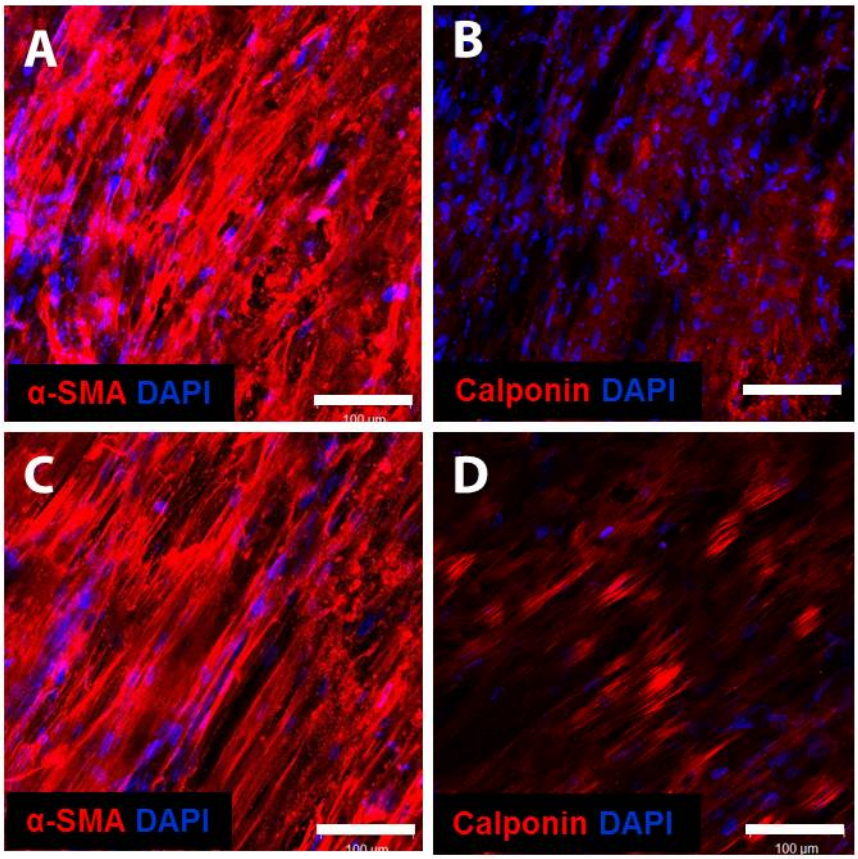

Fig S3: Contractile protein expression of endothelialized TEBVs made with hNDFs or hMSCs matured for 5 weeks under perfusion. TEBVs made with hNDFs express α-SMA (**A**) and calponin (**B**). TEBVs made with hMSCs substantially increase production of α-SMA (**C**) and calponin (**D**) during 5 weeks of perfusion at physiological flow rates. All scale bars indicate 100 µm.

Table S1: TEBV Collagen Fiber Density

Initial CFD (%)	Water Loss (%)	Final CFD (%)	Fold Increase in Collagen Density
0.23 ± 0.01	96.2 ± 0.8	6.2 ± 1.2	26.1 ± 5.9

Table S2: Primers for RT-qPCR.

Target (Gene)	Primer Sequence
ICAM-1 (ICAM1)	Fwd: 5'-CAC CCT AGA GCC AAG GTG AC-3' Rev: 5'- GGG CCA TAC AGG ACA CGA AG-3'
α-SMA (ACTA2)	Fwd: 5'-GAC CTT TGG CTT GGC TTG TC-3' Rev: 5'-GTG CGG ACA GGA ATT GAA GC-3'
Calponin1 (CNN1)	Fwd: 5'-AGG TTA AGA ACA AGC TGG CCC-3' Rev: 5'-ATG AAG TTG TTG CCG ATG CG-3'
B2-microglobulin (B2M)	Fwd: 5'-GGC TAT CCA GCG TAC TCC AAA G-3' Rev: 5'-CAA CTT CAA TGT CGG ATG GAT G-3'

Table S3: Human Vasoactivity Responses

Drug	Target	EC_{50}	Reference
Phenylephrine	Human saphenous vein	10^{-5} M	(*2*)
Acetylcholine	Human brachial artery	0.537×10^{-6} M	(*3*)
Theophylline	Human dorsal hand vein	84 μg/min	(*4*)
Caffeine	Human mammary artery	10^{-6} M	(*5*)

Table S4: Antibodies for Flow Cytometry

Antigen	Conjugate	Source	Isotype	Vendor	Clone
CD31	FITC	Mouse	IgG1, κ	BioLegend	WM59
CD144	PE	Mouse	IgG2a, κ	BioLegend	BV9
CD14	FITC	Mouse	IgG1, κ	BioLegend	HCD14
CD45	FITC	Mouse	IgG1, κ	BioLegend	HI30
CD115	PE	Rat	IgG1, κ	BioLegend	9-4D2-1E4
Mouse IgG1	FITC	Rat	IgG	BioLegend	RMG1-1

Table S5: Antibodies for Immunofluorescence

Type	Antigen	Conjugate	Source	Clonality	Isotype	Vendor	Number
1°	vWF	--	Rabbit	Polyclonal	IgG	Abcam	ab6994
1°	α-SMA	--	Rabbit	Polyclonal	IgG	Abcam	ab5694
1°	Calponin	--	Rabbit	Monoclonal	IgG	Abcam	ab46794
1°	Fibronectin	--	Mouse	Monoclonal	IgG1	Abcam	ab26245
1°	Collagen IV	--	Rabbit	Polyclonal	IgG	Abcam	ab6586
1°	Laminin	--	Rabbit	Polyclonal	IgG	Abcam	ab91006
1°	VCAM-1	--	Mouse	Monoclonal	IgG1	Santa Cruz	sc-13160
1°	E-selectin	--	Rabbit	Polyclonal	IgG	Santa Cruz	sc-14011
1°	ICAM-1	--	Mouse	Monoclonal	IgG1	Santa Cruz	sc-107
1°	Rabbit IgG	--	Rabbit	--	IgG	Life Technologies	02-6102
2°	Mouse IgG	Alexa Fluor 488	Goat	Polyclonal	IgG	Life Technologies	A-11001
2°	Rabbit IgG	Alexa Fluor 594	Goat	Polyclonal	IgG	Life Technologies	A-11012

Supplementary References

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